CERCOSPORA PARTHENIPHILA: A SUITABLE FUNGAL BIOCONTROL AGENT FOR PARTHENIUM WEED

[1]Dr. S. A. Khan and [2]Prof. K. R. Aneja

[1]Principal, Bhagwati College of Management & Technology, Siwaya, Meerut

[2]Deptt., of Microbiology, Kurukshetra University, Kurukshetra, (Haryana)

ABSTRACT

Congress grass (*Parthenium hysterophorus* L.) of family Asteraceae, is labeled as serious weed of wastelands, industrial areas, lawns, orchards, agricultural fields, overgrazed pastures, playgrounds, roadsides, railway tracks, and residential plots and considered as a weed of global significance. Various problems of human health, agriculture, livestock production and biodiversity are posed by this weed in Asia, Africa, Australia and the Pacific. Various methods like physical, chemical and bioherbicidal have been tried to control this weed around the globe. A series of extensive surveys were made to find out nature's bestendemic fungal biocontrol agent of this obnoxious weed. A leaf spot disease creating epiphytotics was regularly observed on *P. hysterophorus*. The organism was isolated from the affected parts of the parthenium by following the standard isolation techniques. On the basis of cultural and morphological characteristics, the isolated pathogen was identified as *Cercospora partheniphila* . Koch's postulates were performed and found satisfactory for the isolated fungus and proved to be pathogenic to this weed as an effective fungal biocontrol agent. Biocontrol efficacy of the fungal suspension was examined on young plants of parthenium weed under injured, uninjured; covered and uncovered conditions.The inoculum was applied to the plants within 2 hrs of sunset to avoid drying and to allow for a natural dew period shortly thereafter. The disease progressed with the increase of the incubation period ultimately resulting in rotting of the complete plants within 22 days of artificial inoculation of the host.

Keywords: *Congress grass, Biocontrol, Cercospora partheniphila, Host-specificity, Injured, Uninjured, Tween 80*

No: Tables: 4 **No: of Figures: 2** **No: of References: 38**

INTRODUCTION

Congress grass (*Parthenium hysterophorus* L.) of family Asteraceae (Tribe: Heliantheae), hereafter referred to as parthenium, is labeled as serious weed of wastelands, industrial areas, lawns, orchards, agricultural fields, overgrazed pastures, playgrounds, roadsides, railway tracks, and residential plots. It is an annual herbaceous weed of Central and South America, and is a global invader that causes severe economic losses in several parts of Africa (McConnachie et al., 2010), Asia (Nath, 1988; Adkins et al. 2005), and Australia (Navie et al., 1996). Parthenium weed was not reported as problematic anywhere till 1970 (Evans, 1997). Australia and India were among the first countries to recognize its invagination. In India, parthenium was introduced accidentally in 1955(Rao, 1956) and since then it has become one of the worst weed of India invade of about 35 million hectare of land(Sushilkumar and Varshney, 2010). It has subsequently invaded most of the sub-continent, including Pakistan(Adkins et al. 2005), Sri Lanka(Jaisurya, 2005) Bangladesh, Nepal (S. Adkins, pers. comm.), southern China and Vietnam(Nath, 1988), Taiwan(Peng et al., 1988), Israel(Joel and Liston, 1986), as well as some Pacific islands(McFadyen, 1992). The weed is notorious in two ways: firstly it is a highly adaptable weed and can grow anywhere, invade all types of pasture lands and cause substantial losses in the yield of agricultural crops(Aneja,1991; Aneja and Khan, 2000). Secondly, parthenin is a predominant allergen present in this weed(Rodriguez, 1975) is responsible for various skin allergies in men and animals besides causing losses to crop productivity and biodiversity (Sushilkumar, 2014). Parthenium is spreading at an alarming rate and not only compete with cultivated crops but also deplete the nutrient pool of the soil in which have they grown (Aneja et al., 2014).

Keeping in view the menace of this weed, attempts have been made to control it.The biological control of weeds by plant pathogens has gained acceptance as a practical, safe, and environmentally beneficial method applicable to agro-ecosystem. The application of plant pathogens comes especially into consideration for parasitic weeds difficult to control via chemical means, or for small-scale and specialized crops where the development of specific chemical solutions is too expensive (Schroeder et al., 1993; Auld and Morin, 1995). Biological control of parthenium weed was first proposed in India during the 1970s and a brief survey was made of the insects attacking the weed in the West Indies. Later, CABI Bioscience (then Commonwealth Institute of Biological Control)was contacted by Australia to assess natural insect enemies in Mexico. The most important event on biological control of this weed in India was reported in 1984, when Indian Institute of Horticultural Research (IIHR), Bangalore imported a chrysomelidae beetle *Zygogramma bicolorata* from Mexico (Jayanth,1987). In 2003, South Africa became the first African country and only the third country worldwide to implement a biological control programme against

the weed. It seems that a suite of agents is needed to achieve effective biological control of parthenium under different environmental conditions.

Present paper deals with the evaluation of biocontrol efficacy of *Cercospora partheniphila* fungus against parthenium weed. The cultural characteristic of *C. partheniphila* was also assessing via analyzing its growth and sporulation on different media at different temperature range under different environmental conditions i.e. light/dark conditions. The host-specific nature of the selected fungus was also determined against some crop plants. The biocontrol potential of the isolated fungus was examined under different experimental conditions i.e. covered / uncovered; injured / uninjured to explore its possibilities as a suitable bioherbicidal (mycoherbicide) agent against young plants of parthenium Host-specific nature of the fungus was also determined in this study.

MATERIALS AND METHODS

Isolation and identification of the biocontrol agent

In order to control this weed by biological means especially with fungal biocontrol agents, surveys were conducted in Haryana, parts of Punjab, Uttar Pradesh and Delhi in search of naturally occurring fungal pathogens on it in different seasons i.e. summer (March to June), rainy (July to October) and winter (November to February) of the year. A leaf spot disease on parthenium was regularly occurring during all seasons. Infected leaves were collected in sterilized polythene bag and brought to the laboratory for study of symptoms, isolation, identification and pathogenicity tests of the pathogen/s involved. Diseased specimen was processed and sent to International Mycological Institute (IMI), Egham, UK for confirmation of the pathogen identification.

The selection of the fungus as a biocontrol agent was done on the basis of disease incidence and intensity in the nature.

$$\%\ Disease\ incidence = \frac{\text{No. of plants infected}}{\text{Total no. of plants counted}} \text{x} 100$$

$$\%\ Disease\ intensity = \frac{\text{Sum of the all numerical disease rating} *}{\text{Total no. of leaves observed x Maximum disease rating}} \text{x} 100$$

* No disease = 0, 1-10% area leaf area affected = 1, 10.1-25% leaf area affected = 2, 25.1-50% leaf area affected = 3, > 50% leaf area = 4

Selection of culture medium and effect of various conditions on the growth and sporulation of biocontrol agent

To determine the best medium for growth and sporulation of fungal biocontrol agent, ten different media were screened. The media used were Potato Dextrose Agar (PDA), Potato Dextrose Agar Plus Yeast extract (PDAY), Parthenium Dextrose Agar (PeDA), Parthenium Dextrose Agar Yeast extract (PeDAY), CzapekDox Agar (CDA), CzapekDox Agar plus Yeast extract (CDAY), Nutrient Agar (NA), V-8 Juice Agar, Potato Sucrose Agar (PSA) and Martin Agar (MA). All the media were sterilized at 15 lb/in^2(121°C) for 15 minutes. Fifteen ml of a given medium was poured into each sterile Petri plates and allowed to solidify. The plates with solidified medium were kept in an inverted position for 24 hrs to remove the thin film of water from the surface. Mycelial discs of 8 mm diameter of the test pathogens, cut from the periphery of seven days old, actively growing colonies, were placed in the centre of each plate. Two sets per medium were prepared to see the effect of light as well as darkness on the growth and sporulation. Out of two sets of inoculated plates, one were kept under florescent light and another under dark (i.e. covered with aluminum foil) and incubated at 25±1°C for 10 days. Three replicates were run per medium per condition for each set. Fungal growth was determined in cm by measuring the diameter of the colony at two places at right angle to each other and an average of the cross diameter was considered as growth of the fungus (Millner, 1977). The sporulation was determined by mounting the number of conidia per ml of the fungal suspension. To determine the effect of temperature on conidial germination, aqueous suspension of the mycelia mat of *C. partheniphila* (6x10^4 conidia + mycelium/ml) were applied in drops onto 2-cm^2 filter paper squared wetted previously with sterile distilled water. Filter paper squares placed on glass microscopic slides were incubated in Petridishes for 14 hrs at 5, 15, 25, 35, 45, and 55°C. Observations were started after 6 hrs incubation. Drops of lactophenol cotton blue were applied to the filter paper surface and per cent germination was determined under 10x and 40x magnifications. For each treatment, three replicates were observed – 90 conidia per replicate.

Evaluation of biocontrol potential of fungal biocontrol agent

To test the biocontrol potential, the fungal biocontrol agent was sub-cultured onto the surface of sterilized Potato Dextrose Agar plus Yeast extract medium in sterilized Petri plates and incubated at 25±1°C for growth and further experiment. The experiment was designed as under.

Parthenium seeds were grown in 24 plastic pots of 2 x 2.5 size with 3 seeds/pot. The pots consisted of sand – soil mixture in the 1:1 ratio. Two sets of pots were prepared. One set without inoculum treatment was kept a control, while on another experimental set, inoculum + surfactant (0.05% Tween 80) was sprayed at four days intervals onto the leaves surfacewhen the plants were at 4-5 leaves

stage by using an atomizer. Spraying was done on two types of plants, i.e. injured/pricked (with sterilized needle) and uninjured/unpricked which were again kept under two different conditions: (i) uncovered; and (ii) covered with polythene bags. The inoculum *C. partheniphila* was prepared on the Potato dextrose agar yeast extract medium, since this medium was found to be the best for growth and sporulation of this fungus. The microbial mass (conidia+mycelium) was harvested by flooding the culture growing on above medium. This liquid microbial mass was transferred into a sterilized Erlenmeyer flask and stirred on a magnetic stirrer for 20-25 minutes. The microbial mass of *C.partheniphila* was adjusted to $6x10^4$ conidia+mycelium/ml by using haemocytometer. The inoculum was sprayed to the young plants of parthenium (3-4 inches in height) within 2 hrs of sunset to avoid drying and to allow for a natural dew period shortly thereafter. Observations were made after four-day intervals for the development of the disease i.e. onset of symptoms and percent area covered by the disease till the death of the parthenium plants.

Evaluation of Host Specificity

Host specificity of the *Cercospora partheniphila* was tested against 10 plant species belonging to the different families Solanaceae, Brassicaceae (Cruciferae), Liliaceae, Asteaceae, Graminae and Papilionaceae. The crop plants were selected on the basis of their economic importance and growth commonly. The plants tested for their host range were tomato (*Lycopersicon esculentum*), lobia (*Phaseolus lunatus*), potato (*Solanum tuberosum*), cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), garlic (*Allium sativum*), onion (*A. cepa*), wheat (*Triticum aestivum*), sunflower (*Helianthus annuus*) and mustard (*Brassica campestris*). The crop plants were grown in plastic pots (3seeds/pot). Growth of *C. partheniphila* fungus was scrapped from the surface of culture medium with sterilized spatula and put into a flask containing sterilized distilled water for the preparation of conidial suspension mixed with 0.5% Tween-80. The plants were injured with sterilized needle and the leaf and stem surfaces inoculated by spraying to run off with the conidial suspension. Control sets were sprayed with sterilized distilled water plus surfactant only. Plants were monitored daily for 2 weeks for studying the symptoms.

RESULTS AND DISCUSSION

On the basis of morphological, cultural and sporulating structures, the isolated pathogen was identified as *Cercospora partheniphila* Chupp and Greene and confirmed by International Mycological Institute (IMI), UK with IMI No. 375238. The symptoms were characterized by the presence of irregular, rounded/oval, light to dark brown sometimes ash centered spots of 1-4 mm diameter mainly restricted to the margins and occasionally the midrib of the leaves of *Parthenium hysterophorus* L. In the T.S. of infected leaf the conidiophores light brown, arising in clusters (5-12 in no.), straight to flexuous, septate, upto 691µm long and 4.5 – 6.8 µm

thick with 1-5 prominent scars. Conidia endohololoblastic, variable in shape and size, filiform, tapering towards the apex with prominent scar at the detachment point, 125 – 480 µm long and 1.5 – 5.7 µm thick.. Colonies greenish black on culture medium (Fig. 1 A,B,C,D)

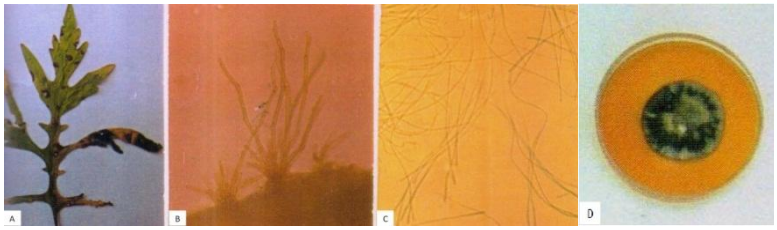

Fig. 1.*Cercosporapartheniphila*A) Leaf spots on parthenium leaf. B) T.S. of infected leaf showing conidiophores, C) Conidia, D) Fungal colony on PDAY medium.

Our findings on culture growth under different conditions revealed that C. *patheniphila* exhibited varying degree of growth on all the ten media tested. C. *partheniphila* showed best growth on CDAY under light followed by MA > PDAY > PDA > PSA >PeDAY> NA > V_8>PeDA>CDA. Under dark, the growth of C. *partheniphila* was recorded best on PDAY followed by PSA>CDAY medium. C. *partheniphila* sporulated best on Potato dextrose agar yeast extract (PDAY) under dark as well as light conditions. A look at the Table 1 reveals that *C. partheniphila* fungus has shown variable results against tested culture media. In case of PDA, PeDA, PeDAY, CDA, CDAY, V_8, MA and NA there was more growth in light as compared to dark, but was not statistically significant in PDA, PeDA. In case of PDAY and PSA the growth was even lesser in light. If we consider both the parameters i.e., growth and sporulation which are the prerequisites of any mycoherbicide for inoculum preparation, *C. partheniphila* should be grown on PDAY either in light and/or dark. Some workers stated that many species grow well in dark, but others require light and some sporulate better under near ultra-violet light(Smith, 1996).

Table. 1 Growth and sporulation of *C. partheniphila* on ten different media after 9 days post incubation

Sr. No.	Media	Growth Diameter(cm)		t value	(Sporulation/ unit area)	
		Dark	Light		Dark	Light
1.	PDA	2.93±0.10	3.12±0.06	1.63	-	-
2.	PDAY	3.63±0.36	2.52±0.10	3.90*	9.33±2.88	8.67±1.44
3.	PeDA	2.28±0.08	2.40±0.09	1.16*	-	-

4.	PeDAY	2.27±0.06	2.82±0.19	2.84*	-	-
5.	CDA	1.88±0.11	2.37±0.08	3.60*	6.00±1.25	5.33±1.19
6.	CDAY	2.95±0.19	3.87±0.19	3.42*	-	-
7.	V_8	2.13±0.02	2.50±0.13	2.81*	3.33±0.72	1.67±0.27
8.	MA	2.65±0.15	3.18±0.01	3.52*	-	2.0±0.47
9.	PSA	2.98±0.05	2.90±0.16	0.47*	-	-
10.	NA	2.51±0.05	3.10±0.11	5.36*	8.33±2.23	-

*Significant at 0.01 level

Of the six temperatures tested to see their effect on conidial germination C. *partheniphila* could germinate only at three temperature i.e. 15°C, 25°C and 35°C while no conidial germination was recorded at 5°C and 45°C. 55°C. 25°C was found to be the best temperature for conidial germination for *C. partheniphila*.

As far as the biocontrol efficacy of selected fungus is concerned, parthenium young plants responded differently towards infection under different conditions i.e. injured/uninjured and covered/uncovered. Artificial spraying of inoculum on the parthenium plants showed the onset of symptoms on leaves, after 3-4 days of inoculation, as pin – point light brown spots on the margins as well as centre of the leaves (Figure.2). The typical disease symptoms were observed on injured (pricked) and uninjured (un-pricked) leaves due to spraying of spore suspension of *C. partheniphila*. The disease progressed with the increase of the incubation period ultimately resulting in rotting of the complete plants within 22 days of artificial inoculation of the host (Table.2).

Fig. 2. Bocontrol potential of *Cercospora partheniphila* **A)**. Set of experiment **B)**. Infection due to fungus on injured and covered plants of parthenium**C).** Leaf spots covering the whole leaf **D).** Complete death of the parthenium plant inoculated *Vs* control.

Infection in covered pots (injured and uninjured 9.34% and 0.60%) that were artificially sprayed with the spore suspension (6x10^4 conidia + mycelia/ml) was higher than in uncovered pots (injured and uninjured 1.36% and 0.09%).Those parthenium leaves were injured on upper surface showed more severe symptoms (Tables. 3 & 4).This suggested a possible role of insects in causing wounds for the entry of the pathogen recorded better control of water hyacinth in the USA when *Cercospora rodmanii* was used in combination with water hyacinth where insects the latter were used to cause injury on leaves (Conway, 1976). Similarly, several others has also been reported that the use of pathogen has resulted in more severe damage to the insect damaged plants because of lower resistance (Freeman and Charudattan, 1984' Charudattan et al., 1978' Charudattan, 1986; Aneja and Singh, 1989). Moreover, parthenium leaves which were injured on upper surface showed high infection both in covered (9.34%) and uncovered (1.36%) pots suggesting a possible role of insects in causing wounds for the entry of the pathogen. The inoculated pathogen was re-isolated thus confirming the pathogenicity of the pathogen to parthenium and usual Koch's postulates.

Table.2. % Infection due to *C. partheniphila* on parthenium leaves after 4,8,12, 16 and 22 days post inoculation

Sr. No.	Days after inoculation	Covered		Uncovered	
		Uninjured	Injured	Injured	Uninjured
1.	4	-	-	-	-
2.	8	0.60±0.16	*1.75±0.50	0.09±0.06	0.21±0.10
3.	12	1.13±0.29	3.31±0.93	0.71±0.11	0.40±0.20
4.	16	3.86±1.00	9.34±2.74	0.60±0.38	1.36±0.64
5.	22	Death of the plants			

*Mean of 12 replicates

Table.3. Statistical analysis of % infection of parthenium leaves by *C. partheniphila* 16 days post inoculation.

Injured		t-value	Uninjured		t-value
Covered	Uncovered		Covered	Uncovered	
9.34±2.74	1.36±0.64	2.83*	0.60±0.15	0.09±0.06	3.04*

*****Significant at 0.5 level

Table.4. Statistical analysis showing the % infection caused by C. *partheniphila*16 days post inoculation between inoculated Vs uninoculated parthenium plants

Treatment	Uninjured		Injured	
	Uncovered	Covered	Uncovered	Covered
Inoculated	0.09±0.06	0.60±0.15	1.36±0.64	9.34±2.74
Un-inoculated	0.06±0.03	0	0.12±0.03	0
t-value	0	4.00*	1.93	3.40*

*****significant at 0.5 level

Higher infection level in the covered pots, may be due to the maintenance of high moisture by the covers which ultimately increased the ability of conidia to germinate and infect. It has been reported that the spray of granular formulation of *Sclerotinia sclerotiorum* (a food based pathogen) permits a mycelia invasion within a few centimeters of the host crown in high humid conditions (Sands and Miller, 1993). Srinivas (1992) also found that, the best results to control water hyacinth (*Eichhornia crassipes*) were obtained when an insect *Neochetina eichhorniae* was used along with the fungal spray of *Cercospora rodmanii*.

The two components of environment viz. temperature and relative humidity play a key role in determining the successful use of plant pathogens as biological control agents. For example, the optimal environmental conditions for the control of sickle pod by *Alternaria cassia* included at least 8 hrs of free moisture at 20 - 30°C (TeBeest, 1991). Region wise field test with this pathogen illustrated the variation in

control caused by differences in environments at the time of inoculation

(Charudattan, 1986). For *Colletotrichum gloeosporioides* f. sp. *aeschynomene* used for *Aeschynomene virginica,* temperature and moisture requirement for rapid disease development occurred rapidly at incubation temperatures ranging from 20-30°C with an optimum near 28°C. Moisture requirement for the establishment of infection included dew period of more than 12 hrs at optimum temperature of 28°C – longer periods being required when dew period temperature were 20, 24 or 32°C (TeBeest et al.,1978). At 36°C, disease development was severely limited. In another study, the plant mortality was greatly influenced by dew period (Mintz et al., 1992). Seedling mortality was 100% after dew period. C. *gloeosporoides* f. sp. *Malvae* controlled *Malva pusilla* if inoculated plants received a dew period of 20 hrs at 20-25°C (Makowski, 1993).

Different crops viz. tomato (*Lycopersicon esculentum*), lobia (*Phaseolus lunatus*), potato (*Solanum tuberosum*), cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), garlic (*Allium sativum*), onion (*A. cepa*), wheat (*Triticum aestivum*), sunflower (*Helianthus annuus*) were tested against C. *partheniphila* for susceptibility or host range study. All the plant species tested were found immune to C. *partheniphila.* In lobia, cabbage and cauliflower where the pathogen was able to penetrate the host but it remained restricted to the penetration site and no symptoms was produced on the host. These results clearly reveal the host – specific nature of C. *partheniphila* against parthenium and could be considered as a suitable biocontrol agent for its development into a mycoherbicide.Literature search revealed that a lot of experiments has been done by the weed scientists to control this weed by fungal biocontrol agent. Some of the workers have isolated different pathogens from parthenium and then tested their biocontrol potential against it. According to Evans (1987), a rust species *Puccinia abrupta* Diet.& Holw.var. *partheniicola* (Jackson) Parmelee was selected for biocontrol of this weed under classical biocontrol strategy. Rust infection hastened leaf senescence, significantly decreased the life span of parthenium weed and also reduced flower production by 90%. Pandey et al. (1992) reported a new collar rot disease of *P. hysterophorus* due to *Sclerotium rolfsii* Sacc. where it was thought to have considerable potential asa mycoherbicide for control of parthenium weed. *Fusarium pallidoroseum* is another important soilborne pathogen reported to be a potential mycoherbicidal agent against parthenium (Kauraw et al., 1997). Beside these, Patel et al. (2015) have discussed various fungal pathogens used as a biocontrol agent against parthenium weed. The fungus *Fusarium oxysporum* PR#12 and *Fusarium solani* PR#13 showed maximum mycoherbicidal potential against parthenium seedlings in sandy soil, 25^0C, 70% moisture and high relative humidity. This fungus was found host specific against parthenium weed. The different species of a soil borne non pathogenic fungi namely *Tricoderma viride* and *Trichoderma virens (=Gliocladium)* have also been used as a suitable biocontrol agent against this weed. Shukla and Pandey (2008) reported very

significant pathogenic diversity in various isolates of *Sclerotium rolfsii* effective against parthenium. Recently, Kaur and Aggarwal (2015) examined biocontrol potential of four deadly strains of *Alternaria macrospora* fungus isolated from parthenium weed. The present paper concluded that various fungal agents have been tried previously to control this weed by biological means but these pathogens were found suffered from one or the other disadvantages like low virulence and wide host range etc. made them unsuitable as candidate for exploitation as a mycoherbicides.

Conclusion

This work aimed for searching a nature's best pathogen which might be highly virulent, host-specific and emerges as an effective mycoherbicide against this weed.Outcomes of this present investigation are: *Cercospora partheniphila* has all the desirable characteristics for its development and exploitation into a mycoherbicide viz. (i) wide distribution and ability to suppress the weed in nature; (ii) host specific nature; (iii) ability to sporulate well on potato dextrose yeast agar (PDAY), a simple and cheap culture medium, within ten days, so that it can be mass produced in a short time, thus making it the best suitable biological control agent for this weed. The findings of the present investigation are preliminary in nature and need not necessarily constitute recommendation for management of this notorious weed. It provided a direction for rigorous investigation in the form of regular survey of complete northern India, isolation of more virulent pathogens capable of bringing mortality of weed rather than damage to foliage alone, enhancing pathogenic capabilities of biocontrol agent by mutation or otherwise which helps in bringing out severe disease outbreak in plant population leading to complete destruction of foliage**.**

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REFERENCES

Adkins S W, Navie S C, Dhileepan K (2005) Parthenium weed in Australia: Research, Progress and Prospects. In proceeding of the Second International Conference on parthenium Management 5-7 December 2005. University of Agricultural Science. Bangolore, India pp. 11-27.

Aneja K R (1991) Deadly weed *Parthenium hysterophorus* L and its control a review. In: Aery N C, Chaudhary B L (eds) Botanical Researches in India Himanshu Publications, Udaipur, pp 258-269.

Aneja K R, Khan SA (2000) Congress grass (*Parthenium hysterophorus* L): Its impact, infestation and biological control with fungal pathogens – An overview. In: Aneja K R, Charaya M U, Aggarwal A K, Hans DK,

Khan S A (eds) Glimpses in Plant Sciences Pargati Parkashan, Meerut, pp 317-322.

Aneja K R, Kumar V, Jiloha P, Kaur M, Sharma C, Surian P, Dhiman R, Aneja A (2014) Potential herbicides: Indian Perspectives. In: Salar R K, Gahlawat S K, Siwach P, Duhan J S (eds) Biotechnology: Prospects and Applications Springer, 197-215.

Aneja K R, Singh K (1989) *Alternaria alternata* (Fr.) Keissler, a pathogen of water hyacinth with biocontrol potential. Tropical Pest Management 35 (4) : 354-356.

Auld B A, Morin L (1995) Constraints in the development of bioherbicides. Weed Technology. 9:638-652.

Charudattan R (1986) Integrated control of water hyacinth (*Eichhornia crassipes*) with a pathogen, insects and herbicides. Weed Sci. 34 : 26-30.

Charudattan R, Perkins B D, Littell R C (1978) Effect of fungi and bacteria on the decline of arthropods damaged water hyacinth (*Eichhornia crassipes*) in Florida. Weed Sci.26 : 101-107.

Conway K E (1976) Evaluation of *Cercospora rodmanii* as a biological control of water hyacinth. Phytopathology66: 914-917.

Evans H C (1997) *Parthenium hysterophorus*: a review of its weed status and possibilities for biological control. Biocontrol News and Information. 18 (3): 89N-98N.

Evans HC (1987) Fungal pathogens of some subtropical andtropical weeds and the possibilities for biological control. Biocontrol News and Information 8: 7–30.

Freeman T E, Charudattan R (1984)*Cercospora rodmanii* Conway - a biological control agent for water hyacinth, Bulletin 842 (technical), Agr. Exp. Sta. Institute of food and Agricultural Science, University of Florida, Gainesville.

Jaisurya AHM (2005) Parthenium weed status and management in Sri Lanka. In: Proceedings of the Second International Conference on parthenium Management, 5-7 December 2005. University of Agricultural Sciences, Bangalore, India :36-43

Jayanth K P (1987) Introduction and establishment of *Zygogramma bicolorata* on *Parthenium hysterophorus* at Bangalore, India. Curr. Sci. 56: 310-311

Joel D M and Liston A (1986) New adventive weeds in Israel. Israel Journal of Botany 35: 215–223.

Kaur M,Aggarwal N K (2015) Biocontrol potential of four deadly strains of *Alternaria macrospora* isolated from parthenium Weed. Plant Pathology Journal 14 (2): 72-78.

Kauraw L P, Chile A, Bhan V M(1997) Evaluation of *Fusarium pallidoroseum* (Cooke) Sacc. for the biocontrol of *Parthenium hysterophorus* L. In: Proceedings of the 1st International Conference on Parthenium Management,

October 6-8, 1997, Dharwad, India, pp 70-74.

Makowski M D (1993) Effect of inoculum concentration, temperature, dew period, and plant growth stage on disease of round-leaved mallow and velvet leaf by *Colletotrichum gloeosporioides* f. sp. *malvae*. Phytopathology83: 1229-1231.

McConnachie AJ, Wstrathie L, Mersie W, Gebrehiwot L, Zewdie K and Abdurehim A (2010) Current and potential geographical distribution of the invasive plant *Parthenium hysterophorus* (Asteraceae) in eastern and southern Africa. Weed Research. DOI: 10.1111/j.1365-3180.

McFadyen R E (1992) Biological control against parthenium weed in Australia. Crop Protection 11: 400–407.

Millner P D (1977) Radial growth responses to temperature by 58 *Chaetomium* species and some taxonomic relationships. Mycologia69: 492-502.

Mintz A S, Heiny D K,Weidemann G J (1992) Factors influencing the biocontrol of tumble pigweed (*Amaranthus albus*) with *Aposphaeria amaranthi*. Plant Dis. 76: 267-269.

Nath R (1988) *Parthenium hysterophorus* L - a review. Agric. Res. 9: 171-179.

Navie S C, McFadyen R E, Panetta F D, Adkins S W (1996) The biology of Australian weeds 27. *Parthenium hysterophorus* L. Plant Protection Quarterly 11, 76–88

Pandey A K, Mishra J, Rajak R C,Hasija S K (1992) Possibility of managing *Parthenium hysterophorus* L. through *Sclerotium rolfsii* Sacc. National Academy Science Letters, India 15: 111–112.

Patel R, Deepika Patel R (2015) Biological Control of Weeds with Pathogens: Current status and Future trends.Int. J. Pharmacy & Life Sciences 6(6):4531-4550

Peng C I, Hu L A, Kao M T (1988) Unwelcome naturalisation of *Parthenium hysterophorus* (Asteraceae) in Taiwan. Journal of Taiwan Museum 41: 95–101.

Rao R S (1956) Parthenium, a new record from India. Journal of Bombay Natural History Society 54: 218-220.

Rodriguez E (1975) Chemistry and distribution of sesquiterpene lactones, flavenoids in parthenium (Compositae): Systemic and ecological implication. Ph.D. Thesis. University of Texas. Austia.

Sands D C, Miller R V (1993) Evolving strategies for biological control of weeds with plant pathogens. Pesticide Sci, 37: 399-403.

Schroeder D, Mueller-Schaerer H, Stinson C S A (1993) A European weed survey in 10 major crop systems to identify targets for biologicalcontrol. Weed Res. 33:449–458.

Shukla R, PandayA K (2008) Pathogenic diversity of *Sclerotium rolfsii* isolates, a potential biocontrol agent against *Parthenium hysterophorus* L. Afr. J.Env. Sci. and Tech. 2 (5): 124-126.

Smith D (1996) Fungi In: Maintaining Cultures for Biotechnology and Industry. Acadmic Press.

Srinivas B (1992) Biological control of some aquatic weeds. Ph. D. Thesis, K. U. Kurukshetra, Haryana (India).

Sushilkumar (2014) Spread, menace and management of Parthenium. Indian J. Weed Sci. 46(3): 205–219.

Sushilkumar, Varshney, J G (2010) Parthenium infestation and its estimated cost management in India. Indian J. Weed Sci. 42(1&2): 73-77.

TeBeest D O (1991) Microbial Control of Weeds. New York: Chapman and Hall.

TeBeest D O, Templeton G E, Smith R J (1978) Histopathology of *Colletrotrichum gloeosporioldes.* Phytopathology 68: 389-393.